J. Pharm. Pharmacol. 1995, 47: 585–587
Received August 16, 1994
Accepted January 11, 1995

Effect of Oleanolic Acid on Complement in Adjuvant- and Carrageenan-induced Inflammation in Rats

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Abstract

The present work was aimed at evaluating the influence of oleanolic acid on complement-related inflammation.

In adjuvant-induced arthritis and carrageenan-induced paw oedema in rats, oleanolic acid was found to possess significant anti-inflammatory and complement-inhibitory activities. The intraperitoneal injection of oleanolic acid (60 mg kg^{-1}, twice a day), before and after Freund's Complete Adjuvant challenge and thereafter repeated for several days, significantly reduced foot-pad thickness of experimental animal models and simultaneously reduced complement activity. Oleanolic acid also produced marked reduction in complement levels and inflammatory effects on carrageenan-induced paw oedema in rats when injected intraperitoneally (60 mg kg^{-1}, twice a day).

Oleanolic acid is a triterpene (3β-hydroxyolean-12-en-28-oic acid), occurring in a large number of plants in the free state, as its acetate and as glycosides in many saponins. It was isolated from seeds of *Luffa cylindrica* (Barua & Bose 1960). Oleanolic acid showed marked anti-inflammatory and anti-arthritic activities in experimental models of inflammation and arthritis (Singh et al 1992) and also inhibits C_3-convertase of the classical complement pathway (Kapil & Sharma 1994). The complement system has been shown to influence each of the factors that comprise the inflammatory response through release of anaphylatoxins (Silva Da Dias & Lepow 1967). In addition to the direct effects of anaphylatoxins, complement activation products elicit the synthesis or secretion of numerous other inflammatory mediators, such as synthesis and secretion of proinflammatory cytokine by macrophages (Bacle et al 1990), by stimulating the platelet release reaction followed by aggregation (Becker et al 1978) and by generating prostaglandins from macrophages (Hadding et al 1982).

It has been observed that complement-induced alterations in the cell surface as well as the interactions of receptors, including complement receptors, adhering to the vascular endothelium enhances inflammation (Frank & Fries 1991). A strong correlation between complement inhibitory and anti-inflammatory activity in experimental models has been observed (Glenn 1966; Noordhock & Bonta 1974). It is also well established that serum complement is activated by carrageenan (DiRosa et al 1971) and levels of activated complement are increased throughout the first 6 h of inflammatory response towards carrageenan.

In this study oleanolic acid is tested for its effect on complement and inflammatory activities in experimental animal models.

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Materials and Methods

Isolation of oleanolic acid

The dried and powdered seeds of *Luffa cylindrica* (2·7 kg) were successively extracted with *n*-hexane and methanol in a Soxhlet extractor. To the concentrated methanolic extract (500 mL) conc. hydrochloric acid (80 mL) was added and the mixture refluxed for 3 h, cooled and filtered. The residue was washed free from acid, dried and extracted with acetone in a thimble Soxhlet. The residue obtained on removal of solvent was crystallized from ethanol to yield oleanolic acid as colourless needles: (5 g) mp 309–310°C, $[\alpha]_D^{25}$ + 81·1°C (C = 0·6 in $CHCl_3$); molecular ion peak (M^+) at m/e 456; acetate, needles from methanol, mp 367–368°C.

Animals

Well-fed and hygienically-maintained male albino Charles Foster rats, 100 g, were obtained from the animal house of the laboratory.

Adjuvant-induced arthritis

Three groups each containing five rats, were innoculated with Freund's Complete Adjuvant (FCA), Sigma Chemical Co. (St Louis, MO, USA). Each rat received a subplantar injection of 250 μL FCA in the midline-mid metatarsal region of the left hind foot-pad. Oleanolic acid and a standard anti-inflammatory drug, ibuprofen (60 mg kg^{-1}, 0·2 mL kg^{-1}) were prepared as fine homogenized suspensions in saline (0·85% NaCl) and were injected intraperitoneally twice daily for five days, 1 h before adjuvant challenge to the two groups of rats; the remaining group of five rats received an equivalent volume of saline and served as control. This dose corresponded to the approximate ED30 for anti-oedemic activity.

Carrageenan-induced paw oedema

Acute paw oedema in rats was induced by injecting 0·1 mL 1% sterile (w/v) carrageenan in saline (Marine Colloid, Div.,

Table 1. Effect of oleanolic acid and ibuprofen on complement and adjuvant-induced arthritis in rats.

Time (h)	Control				Oleanolic acid				Ibuprofen			
	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness (mm)	(%)	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness[b] (mm)	(%)	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness[b] (mm)	(%)
0	0·880 ± 0·008	—	2·574 ± 0·007	—	0·873 ± 0·003	—	2·540 ± 0·049	—	0·892 ± 0·006	—	2·519 ± 0·005	—
24	1·372 ± 0·004	55	5·219 ± 0·008	102	1·220 ± 0·002	39	4·840 ± 0·008	90	1·286 ± 0·010	44	4·793 ± 0·009	90
48	1·490 ± 0·004	69	5·498 ± 0·006	113	1·104 ± 0·004	26	4·400 ± 0·009	73	1·147 ± 0·009	28	4·410 ± 0·006	75
72	1·620 ± 0·005	84	5·730 ± 0·008	122	0·954 ± 0·003	9	4·290 ± 0·009	68	0·986 ± 0·002	10	4·313 ± 0·007	71
96	1·466 ± 0.010	66	5·220 ± 0·010	102	0·908 ± 0·005	3	4·216 ± 0·004	65	0·917 ± 0·003	2	4·214 ± 0·004	67

[a]Mean ± s.d., n = 5, [b]mean ± s.d., from triplicate analysis.

Springfield, USA) into the subplantar region of the left hind-paw (Winter et al 1962). Oleanolic acid and standard anti-inflammatory drug ibuprofen in saline were injected intraperitoneally (60 mg kg^{-1}, 0·2 mL kg^{-1}) to the two groups of five rats, respectively, twice a day, 30 min before and 3 h after carrageenan challenge; the third group of five rats received saline and served as control.

Inflammatory test

The thickness of the left hind-foot was measured just before and over a period of five days for the adjuvant challenge and at different intervals of time for the carrageenan-induced paw oedema, using a sliding vernier scale (Kweifio Okai 1991). Data are expressed as percent increase compared with pre-injection values.

Complement test

Rats were bled immediately before and after injection of test samples and adjuvant, and thereafter bled daily for five days. Carrageenan-induced paw oedema rats were bled before and after the injection of test samples and carrageenan at different intervals of time on the same day. Complement activity and immunohaemolysing effect of the test samples via the classical pathway was determined spectrophotometrically (Kapil & Moza 1992).

Veronal buffer (25 mM, pH 7·3, containing 0·15 mM Ca^{2+} and 0·5 mM Mg^{2+}) was used as diluent in the complement assay and rat serum was used as the source of complement. Sensitized sheep erythrocytes were incubated with the complement of treated and control rats, respectively. Degree of haemolysis was determined spectrophotometrically at 413 nm and then compared with the measured thickness of the rats' left-hind paws, respectively.

Statistics

All results are expressed as the mean ± s.d. Correlation coefficients between complement activity and inflammatory response were calculated in all experiments. Significance of correlation was determined by *t*-value.

Results

Correlation between adjuvant-induced arthritis and complement activity

A strong correlation between adjuvant-induced inflammation and complement activity was observed. Table 1 shows that in the control group adjuvant increases the thickness of the left hind foot-pad by 122% and complement activity by 84% at 72 h. After that there was a gradual decrease in complement activity as well as in foot-pad thickness of rats.

The intraperitoneal injection of oleanolic acid and ibuprofen twice daily for five days produced a significant reduction in inflammation as well as in complement activity.

Oleanolic acid produced a marked effect on complement activity as well as on inflammation; 72 h after injection an increase of only 68% in foot-pad thickness and a 9% rise in complement activity was observed.

Ibuprofen markedly reduced complement activity and inflammation. Seventy-two hours after injection, there was only a 71% rise in inflammation and a 10% rise in complement activity. After 96 h, there was a further decrease in complement activity and inflammation.

Correlation between carrageenan-induced oedema and complement activity

Both inflammation and complement activity were found to increase as a function of time after administration of

Table 2. Effect of oleanolic acid and ibuprofen on complement and carrageenan-induced arthritis in rats.

Time (h)	Control				Oleanolic acid				Ibuprofen			
	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness (mm)	(%)	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness[b] (mm)	(%)	Complement[a] activity (O.D. at 60 min)	(%)	Foot-pad thickness[b] (mm)	(%)
0	0·901 ± 0.016	—	2·260 ± 0·110	—	0·916 ± 0·014	—	2·330 ± 0·065	—	0·924 ± 0·012	—	2·530 ± 0·111	—
3	1·275 ± 0·013	41	3·830 ± 0·111	69	1·181 ± 0·011	28	3·010 ± 0·110	29	1·162 ± 0·009	25	3·380 ± 0·065	33
6	1·460 ± 0·013	62	4·600 ± 0·095	103	1·346 ± 0·006	49	3·350 ± 0.097	43	1·284 ± 0·014	38	3·500 ± 0·100	38
24	1·171 ± 0·008	29	3·720 ± 0·132	64	1·121 ± 0·006	24	2·630 ± 0·140	12	1·101 ± 0·013	19	2·840 ± 0·127	12

[a]Mean ± s.d., n = 5, [b]mean ± s.d., from triplicate analysis.

carrageenan and reached maxima at 6 h. Table 2 shows that in the control group, rat-paw oedema and complement activity were increased maximally by 103 and 62%, respectively, 6 h after injection. No significant rise in complement activity or in inflammation was observed in treated rats. Six hours after carrageenan injection, only a 49% rise in complement activity and a 43% rise in paw oedema was observed in rats treated with oleanolic acid.

In the case of ibuprofen-treated rats only a 38% rise in paw oedema formation and a 38% increase in complement activity was observed, 6 h after carrageenan injection.

Discussion

The present study was undertaken primarily to study the complement-related anti-inflammatory effect of oleanolic acid. Although inflammation is a multimediated process (Willoughby 1970), it has been observed that complement components play a key role in inflammation (Frank & Fries 1991).

We observed that when inflammation appears complement activity increases in both the adjuvant-induced arthritis and carrageenan-induced paw oedema in rats, but, when oleanolic acid and the standard anti-inflammatory drug, ibuprofen, were tested for their complement-related anti-inflammatory activities, no significant rise in complement activity or in inflammation was observed in either of the experimental models of inflammation. A significant reduction in adjuvant-induced arthritis and in complement activity was observed simultaneously 72 h after adjuvant challenge. In carrageenan-induced paw oedema, marked reduction in complement and inflammatory activities of oleanolic acid and ibuprofen were observed, respectively.

Oleanolic acid has been found to possess an inhibitory effect on complement (Kapil & Sharma 1994) and anti-inflammatory activities in both the adjuvant-induced arthritis and carrageenan-induced paw oedema in rats (Singh et al 1992). The results are consistent with our previous observations of oleanolic acid which show inhibition of C_3-convertase of the classical complement pathway in-vitro (Kapil & Sharma 1994). The present study is in agreement with previous reports of Englberger et al (1988) on Rosamarinic acid, and of Kapil & Moza (1992) and Kapil (1994) on boswellic acids, which showed that these acids inhibit C_3-convertase, complement activation and complement-dependent inflammation. As it is evident that complement plays a key role in inflammation (DiRosa et al 1971; Sell 1980) it would be predicted that inhibition of complement activity might be a possible mechanism for inhibiting inflammatory increase. The inhibition of complement activity inhibits release of anaphylactic peptides and other chemotactic factors with the result that migration of leucocytes is inhibited and no inflammatory response is observed (Jensen 1967).

Our results indicate that oleanolic acid may offer promise for the therapy of inflammation and other disorders associated with complement activation.

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